

Claims

1. Nucleic acid molecule comprising a heat-inducible promoter and selected from the following nucleic acids:
 - (a) a nucleic acid the sequence of which comprises the promoter sequence of a *Hansenula polymorpha* gene coding for a protein with trehalose-6-phosphate synthase activity;
 - (b) a nucleic acid with the sequence indicated in SEQ ID NO:1;
 - (c) a nucleic acid having a sequence which exhibits at least 40% identity over a length of 300 bp with one of the sequences indicated in (a) or (b);
 - (d) a nucleic acid which hybridizes to the complementary strand of one of the nucleic acids indicated in (a), (b) or (c);
 - (e) a derivative of one of the nucleic acids indicated in (a), (b) or (c) obtained by substitution, addition and/or deletion of one or more nucleotides;
 - (f) a fragment of one of the nucleic acids indicated in (a) to (e) which retains the function of the heat-inducible promoter;
 - (g) a combination of several of the nucleic acids indicated in (a) to (f), wherein the sequences of the nucleic acids may be different or the same;

or

a nucleic acid molecule having a sequence complementary to the sequence of one of the nucleic acids indicated in (a) to (g).

2. Nucleic acid molecule according to Claim 1, **characterized in that** the nucleic acid indicated under (c) exhibits at least 60% identity with one of the sequences indicated in (a) or (b), or the complementary sequence thereof.

3. Nucleic acid molecule according to Claim 1, **characterized in that** the nucleic acid indicated under (c) exhibits at least 80% identity with one of the sequences indicated in (a) or (b), or the complementary sequence thereof.
4. Nucleic acid molecule according to Claim 1, **characterized in that** the nucleic acid indicated under (c) exhibits at least 90% identity with one of the sequences indicated in (a) or (b), or the complementary sequence thereof.
5. Nucleic acid molecule according to Claim 1, **characterized in that** the nucleic acid indicated under (c) exhibits at least 95% identity with one of the sequences indicated in (a) or (b), or the complementary sequence thereof.
6. Nucleic acid molecule according to one of the preceding claims, **characterized in that** it exhibits at least one heat shock element having the sequence
NGAANNNNNNNGAAN (SEQ ID NO:2) or the complementary sequence thereof,
wherein the nucleotides denoted by N may be A, T, C or G independent of each other.
7. Nucleic acid molecule according to claim 6, **characterized in that** it exhibits at least one heat shock element with the sequence NGAANNBWMNNGAAN (SEQ ID NO:3) or the complementary sequence thereof, wherein B is a G, C or T, W an A or T, and M a C or A.
8. Nucleic acid molecule according to Claim 7, **characterized in that** the heat shock element is selected from TGAAGCCTCTTGAAA (SEQ ID NO:4) and/or
TGAATATAAAGGAAA (SEQ ID NO:5) and/or the complementary sequences thereof, wherein two or more heat shock elements, where present, may exhibit the same or different sequences.
9. Nucleic acid molecule according to claims 6, 7 or 8, **characterized in that** it exhibits at least two different heat shock elements.

10. Nucleic acid molecule according to one of the preceding claims, **characterized in that** it contains no STRE element having the sequence CCCCT or AGGGG.
11. Nucleic acid molecule according to Claim 1, **characterized in that** the fragment indicated under (f) comprises the sequence from nucleotide 228 to nucleotide 792 in SEQ ID NO:1.
12. Nucleic acid molecule according to Claim 1, **characterized in that** the fragment indicated under (f) comprises the sequence from nucleotide 492 to nucleotide 792 in SEQ ID NO:1.
13. Nucleic acid molecule according to Claim 1, **characterized in that** the fragment indicated under (f) comprises the sequence from nucleotide 627 to nucleotide 713 in SEQ ID NO:1.
14. Nucleic acid molecule according to one of the preceding claims, **characterized in that** it further comprises at least one nucleic acid sequence for a heterologous gene under the transcriptional control of the heat-inducible promoter .
15. Nucleic acid molecule according to one of Claims 1 to 13, **characterized in that** it further comprises a nucleic acid sequence under the transcriptional control of the heat-inducible promoter which is selected from the following sequences:
 - (i) a nucleic acid sequence which encodes a polypeptide with the amino acid sequence of the trehalose-6-phosphate synthase of *Hansenula polymorpha*;
 - (ii) a nucleic acid sequence as indicated in SEQ ID NO:6;
 - (iii) a nucleic acid sequence which exhibits at least 80% identity with the sequence indicated in SEQ ID NO:6;
 - (iv) a nucleic acid sequence which encodes a polypeptide with the amino acid sequence indicated in SEQ ID NO:7 or with a partial sequence thereof, wherein the polypeptide exhibits trehalose-6-phosphate synthase activity;

- (v) a nucleic acid sequence which in consideration of the degeneration of the genetic code would encode a polypeptide with the amino acid sequence indicated in SEQ ID NO:7 or with a partial sequence thereof, wherein the polypeptide exhibits trehalose-6-phosphate synthase activity;
 - (vi) a nucleic acid sequence which encodes a polypeptide the amino acid sequence of which exhibits at least 80% identity with the amino acid sequence indicated in SEQ ID NO:7.
16. Nucleic acid molecule according to Claim 15, **characterized in that** the nucleic acid sequence indicated under (iii) exhibits at least 90% identity with the sequence indicated in SEQ ID NO:6.
 17. Nucleic acid molecule according to Claim 15, **characterized in that** the nucleic acid sequence indicated under (vi) encodes a polypeptide the amino acid sequence of which exhibits at least 90% identity with the amino acid sequence indicated in SEQ ID NO:7.
 18. Host cell containing a nucleic acid molecule according to one of Claims 1 to 17, wherein the host cell is a prokaryotic or eukaryotic cell.
 19. Host cell according to Claim 18, **characterized in that** the eukaryotic cell is a fungal cell.
 20. Host cell according to Claim 19, **characterized in that** the fungal cell is a yeast cell.
 21. Host cell according to Claim 20, **characterized in that** the yeast cell is *Hansenula polymorpha*.
 22. Expression vector comprising at least one nucleic acid molecule according to one of Claims 1 to 13.

23. Expression vector comprising at least one nucleic acid molecule according to one of Claims 14 to 17.
24. Kit, comprising:
 - (a) an expression vector according to Claim 22, which is suitable for having cloned into it a nucleic acid which encodes a recombinant protein, and
 - (b) a host cell suitable for induction of the heat-inducible promoter and for production of the recombinant protein.
25. Kit, comprising:
 - (a) an expression vector according to Claim 23 and
 - (b) a host cell which is suitable for induction of the heat-inducible promoter and for production of a protein encoded by a coding sequence under the transcriptional control of the heat-inducible promoter.
26. Use of a nucleic acid molecule according to one of Claims 1 to 17 or of a host cell according to one of Claims 18 to 21 or of an expression vector according to Claims 22 or 23 or of a kit according to Claim 24 or 25 for expression of a gene under the control of the heat-inducible promoter.
27. Use of a nucleic acid molecule according to one of Claims 1 to 17 or of a host cell according to one of Claims 18 to 21 or of an expression vector according to Claims 22 or 23 or of a kit according to Claim 24 or 25 for the production of one or more proteins.
28. Method for the production of one or more proteins, comprising
 - (i) Cloning of at least one nucleic acid which encodes a recombinant protein into an expression vector according to Claim 22, such that the nucleic acid thus cloned is under the transcriptional control of the heat-inducible promoter;

- (ii) introduction of the expression vector obtained in (i) into a host cell suitable for induction of the heat-inducible promoter and for production of the recombinant protein;
- (iii) cultivation of the host cell obtained in (ii);
- (iv) induction of the heat-inducible promoter by methods known per se.

29. Method for the production of one or more proteins, comprising

- (i) introduction of an expression vector according to Claim 23 into a host cell suitable for induction of the heat-inducible promoter and for production of the recombinant protein;
- (ii) cultivation of the host cell obtained in (i);
- (iii) induction of the heat-inducible promoter by methods known per se.

New Claim 1:

1. Nucleic acid molecule comprising a heat-inducible promoter and selected from the following nucleic acids:
 - (a) a nucleic acid the sequence of which comprises the promoter sequence of a *Hansenula polymorpha* gene coding for a protein with trehalose-6-phosphate synthase activity;
 - (b) a nucleic acid with the sequence indicated in SEQ ID NO:1;
 - (c) a nucleic acid having a sequence which exhibits at least 40% identity over a length of 300 bp with one of the sequences indicated in (a) or (b);
 - (d) a nucleic acid which hybridizes with the complementary strand of one of the nucleic acids indicated in (a), (b) or (c);
 - (e) a derivative of one of the nucleic acids indicated in (a), (b) or (c) obtained by substitution, addition and/or deletion of one or more nucleotides;
 - (f) a fragment of one of the nucleic acids indicated in (a) to (e) which retains the function of the heat-inducible promoter;
 - (g) a combination of several of the nucleic acids indicated in (a) to (f), wherein the sequences of the nucleic acids may be different or the same;

or

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a nucleic acid molecule having a sequence complementary to the sequence of one of the nucleic acids indicated in (a) to (g),

with the proviso that the nucleic acid molecule does not exhibit the promoter sequence of the trehalose-6-phosphate synthase gene of *Saccharomyces cerevisiae* or *Schizosaccharomyces pombe*.

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New Claim 18:

18. Host cell not occurring naturally and containing a nucleic acid molecule according to one of Claims 1 to 17, the host cell being a prokaryotic or eukaryotic cell.

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